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PRINCIPAL INVESTIGATOR: Jeri S. Janowsky, Ph.D.

CONTRACTING ORGANIZATION: Oregon Health and Science University
Portland, OR 97239-3098

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Jeri S. Janowsky

Email: janowskj@ohsu.edu

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14. ABSTRACT
Androgen deprivation is associated with an increased risk for neurodegeneration in both animal models and humans. Therefore the overall objective of this research program is to understand whether androgen deprivation therapy (ADT) decreases the quality of survival by amplifying age-related cognitive decline and increasing the risk for neurodegenerative disease. We focus in this report on findings of brain white matter degeneration using diffusion tensor imaging. We found that men on ADT or had ever used ADT had reduced prefrontal white matter integrity as compared to healthy elderly. In addition, white matter integrity in men on ADT was not related to age whereas it declined with increasing age in healthy men as well as in men with prostate cancer who are not on ADT. The findings were specific; prefrontal white matter was affected by ADT treatment but not anterior or posterior callosum. The diffusivity data suggests the changes are due to axon drop out not loss of myelin as there were no treatment effects on transverse diffusivity. We hypothesize that ADT may accelerate cortical brain aging, followed by relative stability in white matter integrity thereafter.

15. SUBJECT TERMS
Prostate cancer, neurodegeneration, white matter, diffusion tensor imaging, neuroimaging

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INTRODUCTION:

The overall objective of this research program is to understand whether androgen deprivation therapy (ADT) decreases the quality of survival by amplifying age-related cognitive decline and increasing the risk for neurodegenerative disease. Memory, induced brain activity using fMRI, and tissue integrity using a variety of neuroimaging methods are being studied in men on long versus short term treatment with ADT as well as men who are not on ADT.

BODY:

The following activities outlined in the Statement of Work have been accomplished in this first year:

1. IRB and Recruitment: We initiated and completed all IRB approvals, renewals, and approval to use OSCaR (Oregon State Cancer Registry) for additional help with recruitment. As of August 1, OSCaR has mailed 1,000 letters to potential research participants, and this resulted in 163 response forms in addition to 21 men from the OHSU hospital and clinics. This is a much better response rate than we had anticipated and although this increases the burden of screening, it bodes well for sufficient subjects to complete the study. Of these respondents, 118 have been screened for the study, 15 enrolled with rest either have exclusions for the study or chose not to participate. OSCaR will continue to send recruitment letters as we complete screenings and the schedule opens for additional subject test visits.
2. Pilot Testing of Protocol and Analysis Tools, and Testing of Subjects: This is completed and testing of research participants is continuing as is analysis of data.
3. Staff Training: This is completed along with hiring and training of a new assistant.
4. Automate Neuroimaging Analysis: This has also been completed and was used to analyze pilot diffusion tensor imaging data (see below)

The study is ongoing and we do not yet have sufficient data to make the key comparisons: effects of short term androgen deprivation versus chronic treatment on indices of neurodegeneration. Therefore, we will focus here on the findings from an analysis of our initial diffusion tensor imaging data (see also Roalf et al. abstract). Note that some of the data was collected during a prior study. This dataset will continue to be expanded with the ongoing study, but we expect to submit a manuscript of these initial findings later this month.

<u>Androgen deprivation results in white matter loss as shown by diffusion tensor imaging</u>

Androgen deprivation is associated with an increased risk for neurodegeneration in both animal models and humans(Moffat et al., 2004; Rosario & Pike, 2008). Therefore, we examined white matter integrity in men on androgen deprivation for treatment of prostate cancer. Diffusion tensor imaging was used to investigate white matter integrity in 13 men with prostate cancer on androgen deprivation therapy(ADT), 15 men who had cancer but were not on ADT, and 15 healthy elderly (HE; See Table 1 for participant characteristics). Fractional anisotropy (FA), and longitudinal and transverse diffusivity were measured in the corpus callosum and prefrontal cortex (PFC). Ever- use versus never use of ADT was also examined as was the relationship between white matter integrity and age.

Analyses of variance (ANOVA) was used to examine treatment group (HE, ADT, Non-ADT) and treatment history (EVER, NEVER, HE) differences with significance at alpha = 0.05. Post-hoc analysis used a Bonferroni correction. Exploratory analyses of hemisphere were conducted as follow-up for the PFC DTI data using ANOVAs. Correlations between FA and age were calculated using Pearson's coefficients.

<u>Results</u>: <u>Effects of Treatment on FA</u>: Current treatment affected FA values in the PFC ($F(2,40)=3.68$, $p=.034$), but not whole brain, genu or splenium (ps> .10). Healthy older men had higher PFC FA values than men on ADT ($p=.04$) but not as compared to Non-ADT ($p>.10$), who did not differ from each other ($p>.10$). The treatment groups differed for left [$F(2,40)=5.12$, $p=.01$], but not right PFC [$F(2,40) =1.55$, $p > .244$]. Post hoc test showed that healthy elderly

had higher left PFC FA values as compared to men on ADT (p=.008) but not as compared to Non-ADT men (p=.16;Figure 1), who did not differ from each other (p>.10).

Differences in Diffusivity**:** Current treatment affected LD values for the PFC [F(2,40)=6.12, p=.005], but not in the genu or splenium (ps> .10). Both ADT (p=.027) and Non-ADT men (p=.008) had reduced PFC LD compared to HE, but they did not differ from each other (p>.10). The treatment groups differed for left PFC LD F(2,40)=6.73, p=.003] and there was a marginal effect for right PFC LD (p=.09). ADT and Non-ADT men had lower left (ps<.01), but not right (ps>.05) PFC LD as compared to HE men, but did not differ from each other (ps>.10). There were no main effects of current treatment status for TD in the genu, splenium, or PFC. In general, the same effects were found for men who had ever used ADT as those currently on treatment reported here.

Correlations between FA and Age: Age was inversely related to FA in the genu (r=-.572, p=.03), splenium (r=-.680, p=.005) and PFC (r=-.575, p=.025), but not whole brain in the HE. This age-related decline was present in left (r=-.520, p=.05) and right (r=-.578, p=.02) PFC. This was also the case for the PFC (r=-.745, p=.001), but not in whole brain, genu or splenium in the NonADT men. Left (r=-.681, p=.005) PFC showed age-related FA decline in Non-ADT men while right (r=-.488, p=.07) PFC showed a marginal age-related decline. Age was not related to FA in men on ADT (ps'> .10). Similar effects were found in the EVER/NEVER analysis. That is, there was no relationship between age and FA in men who had EVER used ADT (p> .10), but FA was inversely related to age in those who had never used ADT (r=-.862, p=.02) in the PFC. No relationships with age were found whole brain, genu or splenium (See Figure 2).

Discussion: To our knowledge, this is the first study assessing the influence of hypogonadism on white matter integrity in elderly men. We found that men on ADT or had ever used ADT had reduced prefrontal white matter integrity as compared to healthy elderly. In addition, white matter integrity in men on ADT was not related to age whereas it declined with increasing age in healthy men as well as in men who are not on ADT. The findings were specific; prefrontal white matter was affected by ADT treatment but not anterior or posterior callosum. The diffusivity data suggests the changes are due to axon drop out not loss of myelin as there were no treatment effects on transverse diffusivity. Overall the men with prostate cancer who were not currently on ADT or had never used ADT had FA values that fell between the HE and men on ADT and did not differ from either. The reason for their small decline in FA is not clear. It did not appear to be due to temporary use of ADT in the past as the results from EVER/NEVER analysis did not differ substantially from the analysis of current treatment, however a larger sample who had briefly used ADT in the past would be needed to further understand whether some aspect of the prostate cancer diagnosis or if even brief use of ADT impacts the brain.

The reduced FA and higher longitudinal diffusivity in those on ADT is not solely accelerated aging. First, prefrontal FA was affected but not the callosum or splenium as is the case in aging. While anterior regions are more likely to show age-related changes than posterior regions, we would have expected the anterior callosum to also be affected if ADT-induced changes followed the pattern of normal aging from our own results, which was similar to age effects in other DTI studies. Second, both myelin and axon loss typify aging as shown by lower axial and higher longitudinal diffusivity, although there is some debate as to whether both myelin and axon drop out are equally affected (Salat et al., 2008; Madden, Bennett, & Song, 2009; Salat et al., 2005). However, the most striking results were the correlations between age and FA values in the three treatment groups. FA declines with increasing age in both healthy elderly and men with prostate cancer who are not on ADT. In contrast there was no such relationship in men on ADT. If ADT-induced white matter degeneration works by the same processes as normal age related decline, then ADT accelerates aging in men on ADT in their 50's and 60's as they have FA values similar to healthy men in their 80's. However, a similar

loss does not occur in an aged brain. That is, men on ADT who were older, had FA values similar to healthy elderly men. One possibility is that ADT first affects prefrontal white matter, but if prefrontal cortex has already declined, such as in the very elderly, no further loss is detected. One possibility then is that a progression of white matter loss in other regions would be found in the very old men on ADT such as parietal or temporal white matter. Alternatively, we don't know if these results are an interaction between factors that predispose the brain to cognitive and neural degeneration such as genetic risk for Alzheimer's disease and the effects of testosterone deprivation late in life. Nor is it yet clear if these changes predispose the men on ADT to neurodegenerative diseases, such as Alzheimer's disease. Instead, ADT may accelerate cortical brain aging, followed by relative stability in white matter integrity thereafter. The on-going cognitive assessments in this study will aid in resolving this issue.

KEY RESEARCH ACCOMPLISHMENTS:

- The potential neurotoxicity of ADT can be studied with neuroimaging and cognitive measures and may permit an understanding of the neurobiology underlying cognitive effects.
- Initial results suggest that ADT induces cortical white matter loss.

REPORTABLE OUTCOMES:

Abstract: Presented 6/09 at Human Brain Mapping Meeting, San Francisco, California.
The Effect of Androgen Deprivation on Prefrontal White Matter
David R. Roalf[1], Yosef A. Berlow, Mahria R. Lebow, David H. Salat, Jeri S. Janowsky

Abstract: To be presented at the Society for Neuroscience Meeting 10/09, Chicago Il.
Prefrontal activity does not reflect androgen deprivation induced memory impairment.
L.A. Young, M.R. Lebow, D.R. Roalf, T.M. Beer, and J.S. Janowsky
Oregon Health & Science University, Portland, OR
Department Behavioral Neuroscience

Manuscript in preparation:
D.R. Roalf, Y.A. Berlow, M.R. Lebow, D.H. Salat, T.M. Beer, J.S. Janowsky
Androgen deprivation results in white matter loss as shown by diffusion tensor imaging

CONCLUSION:

We cannot yet reach conclusions on the issue of short versus long-term ADT treatment and risk for neurodegeneration. However, the initial DTI data presented above, suggests that white matter in the brain is affected by ADT and that the character of the decline is not simply accelerated aging.

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APPENDICES
See attached abstracts

SUPPORTING DATA

Figure 1

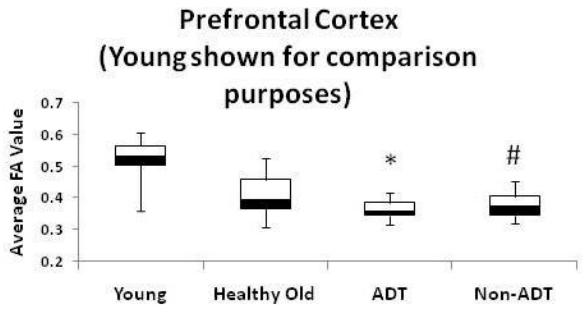
Young > Healthy, ADT, Non-ADT

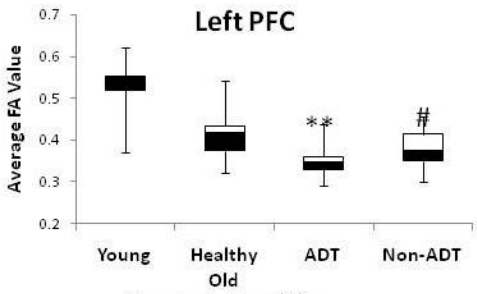
* p=.02

\# p=.09

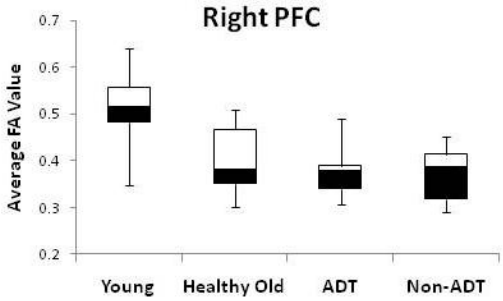
** p=.005

as compared to Healthy Old



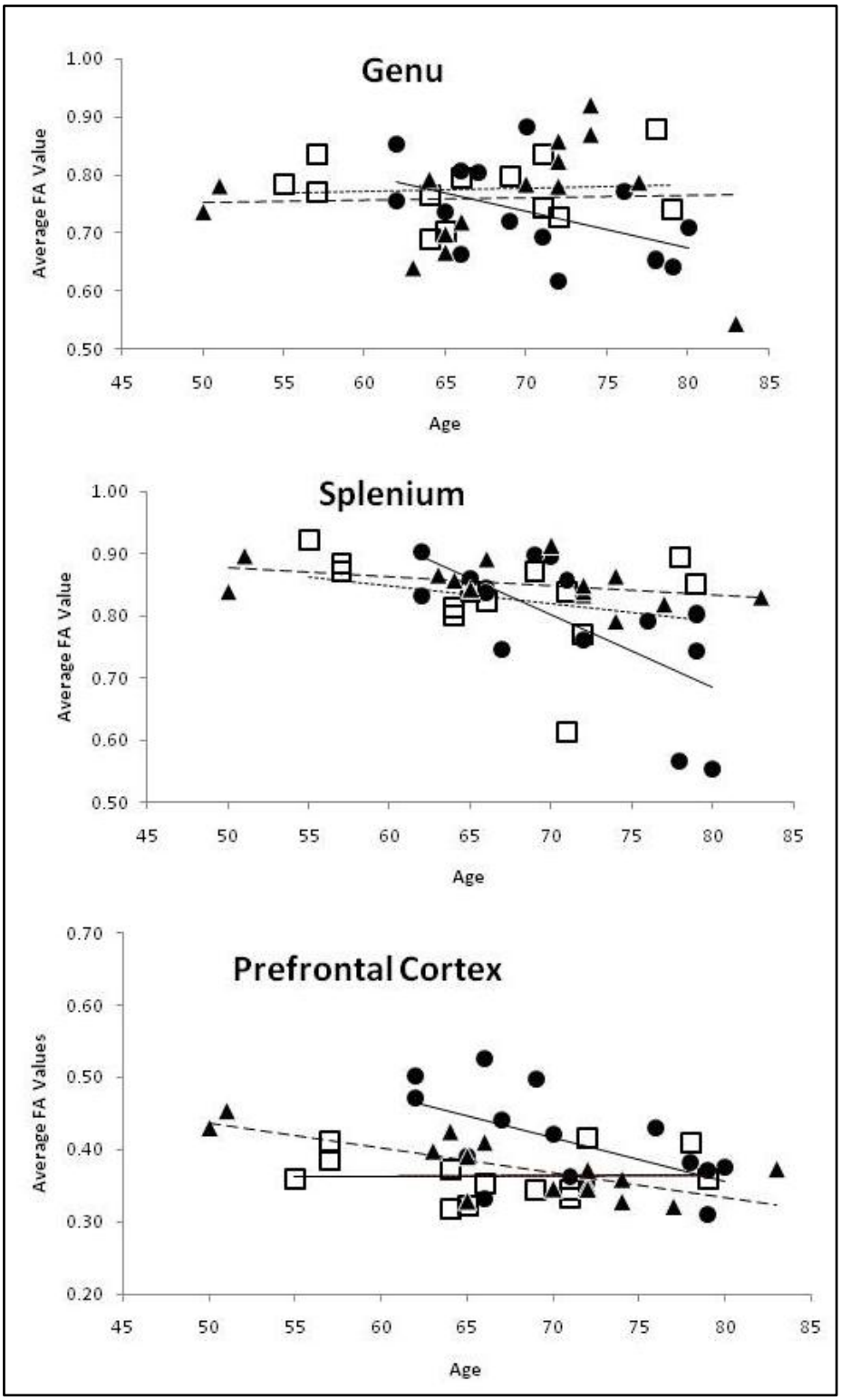


Young > Healthy, ADT, Non-ADT



Young > Healthy, ADT, Non-ADT

Figure 2


Genu
Average FA Value
1.00
0.90
0.80
0.70
0.60
0.50
45
50
55
60
65
70
75
80
85
Age
Splenium
Average FA Value
1.00
0.90
0.80
0.70
0.60
0.50
45
50
55
60
65
70
75
80
85
Age
Prefrontal Cortex
Average FA Values
0.70
0.60
0.50
0.40
0.30
0.20
45
50
55
60
65
70
75
80
85
Age

Abstract: Presented 6/09 at Human Brain Mapping Meeting, San Francisco, California.

The Effect of Androgen Deprivation on Prefrontal White Matter

David R. Roalf[1], Yosef A. Berlow, Mahria R. Lebow, David H. Salat, Jeri S. Janowsky
[1]Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, Oregon;
[2]Athinoula A Martinos Center for Biomedical Imaging, MGH Dept. Radiology, Charlestown MA

Introduction: Androgens play an important role in neuroprotection and low testosterone is a risk factor for neurodegenerative disease in men (1). However, there is little direct neuroimaging data examining the effects of androgen loss on the brain. Androgen deprivation therapy (ADT) is commonly used to delay the progression of prostate cancer (PC), but neurocognitive side effects such as memory loss are often reported (2) and the pathophysiological source of the memory loss is not known.

Methods: The integrity of white matter was examined using Diffusion Tensor Imaging (DTI) in men with PC using ADT (N= 10), men with PC not receiving ADT (NON-ADT; n=15) and in age- and gender-matched controls (HE; n=15). Fractional anisotropy (FA) was measured in the corpus callosum (genu and splenium) and prefrontal white matter. Diffusion tensors were modeled at each voxel to create FA maps. The resulting FA maps were thresholded to include FA values between 0.2-1.0 (3) and white matter mask images were generated from these thresholded FA maps. Non brain voxels were removed from these thresholded FA maps using brain masks generated from the first b0 image (4). Rectangular regions-of-interest (ROI;125 mm^3) were placed on single axial slices in the center of the genu and splenium using the thresholded FA map as a reference. Square ROIs (7.5 mm x 7.5 mm) were placed bilaterally within the PFC white matter on three contiguous axial slices (422 mm^3), with the middle slice located at the level of the center of the genu identified using orthogonal views (See Figure). ROIs were positioned diagonal to the frontal horns of the lateral ventricles in the center of the deep frontal white matter using both the white matter masking image and the b0 image as references. All ROIs were then superimposed on individual FA maps from which the FA values for each ROI were calculated. Memory performance was evaluated using word list learning. FA values were related to word list memory performance.

Results: There were no group differences in the corpus callosum FA values. However, prefrontal white matter FA values were significantly higher in healthy older men than men on ADT ($p<.02$) and marginally higher than NON-ADT men ($p=.08$). In particular, left prefrontal cortex had higher FA values in HE and NON-ADT men than men on ADT ($p<.001$, $p=.098$ respectively; see Figure). While memory performance was positively related to white matter FA measures in healthy men (ps< .07), it was not related to memory performance in men with prostate cancer.

Conclusion: This information suggests that testosterone has a neuroprotective role for white matter in aging but other effects of PC treatment need to be examined to address small but significant changes in NON-ADT men. This information is clinically important for decisions regarding when to initiate ADT in men with prostate cancer.

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Abstract: To be presented at the Society for Neuroscience Meeting 10/09, Chicago Il.

Prefrontal activity does not reflect androgen deprivation induced memory impairment.

L.A. Young, M.R. Lebow, D.R. Roalf, T.M. Beer, and J.S. Janowsky
Oregon Health & Science University, Portland, OR
Department Behavioral Neuroscience

Androgens have both neuromodulatory and neuroprotective effects on the brain. Androgen deprivation is a common treatment to slow the progression of prostate cancer, and anecdotal reports by men treated with androgen deprivation therapy (ADT), as well as some clinical studies, report neurocognitive side effects such as memory impairments. The mechanism of these side effects is not known. Therefore we examined memory and used functional magnetic resonance imaging (fMRI) to examine brain activity in *a priori* regions of interest; the left inferior frontal gyrus (LIFG) and the medial frontal gyrus (MFG). Men with prostate cancer currently on ADT (n=15) were compared to men with prostate cancer not currently on ADT (NONADT n=15) and healthy men of the same age (HEALTHY n=15). Verbal memory was assessed using the paragraph recall test and fMRI imaging took place during encoding of a word list. Memory for both tasks was assessed immediately and after a 30-minute retention interval. Men on ADT had marginally worse word list memory after the retention interval than healthy men (p=.05) and NONADT men (p=.09), who did not differ from each other. Paragraph recall scores were lower for ADT men, but with our small sample size we did not find significant group differences. When we combined the data with previous data from our lab (Beer et al., 2006), men on ADT (ADT N= 46; NONADT n= 23, HEALTHY n= 35) also have worse paragraph memory ADT < NONADT = HEALTHY (p< .02) after the retention interval. The significant loss of memory was not accompanied by group differences in prefrontal signal change during encoding in either MFG or LIFG (ps'> .10). Nor was percent signal change at encoding related to subsequent memory performance as a whole or within any group, with the exception that signal change for remembered words at the 30 minute retention interval was greater in NONADT than HEALTHY and ADT men in the LIFG (ps' < .05). These findings suggest that while ADT results in verbal memory impairments, failure of prefrontal activity at encoding is not the mechanism that underlies the deficit. Future studies that examine medial temporal lobe structures and those that examine the time course of ADT effects may elucidate the underlying mechanisms of ADT induced memory impairment.

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